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Gonad Response to Calcium and a Comparison of the Effects of Calcium, Potassium, Acetylcholine and γ-Aminobutyric Acid on the Sea Urchin Gonad

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ABSTRACT—Gonad response to calcium and the effects of calcium, potassium, acetylcholine (ACh) and γ-aminobutyric acid (GABA) on the gonads of the sea urchin, *Strongylocentrotus intermedius* were studied on the basis of measurements of the potential difference (PD). Rhythmical contraction due to Ca^{2+} occurred at a concentration exceeding 50 mM and its frequency increased as the concentration rose above this. In mature gonads, rhythmical contraction lasted for over an hour. Among four stimuli, ACh initiated threshold-contraction at the lowest concentration, 10^{-8} or 10^{-7} M. In relative response (RR) indicating the PD of a certain stimulation against that of 500 mM K^+, the RR of GABA and K^+ reached 1.0 but Ca^{2+} and ACh saturated near 0.8. Ca^{2+} and GABA induced both rhythmical and smooth phasic contraction while K^+ and ACh, only smooth long lasting contraction. The response to 50 mM K^+ and 50 mM Ca^{2+} persisted for more than an hour but that toward 2 mM GABA and 2 mM ACh, only about 20 min.

INTRODUCTION

Palmer [1] indicated that, on dropping potassium chloride directly onto a gonad surface, visible steady contraction of the gonad wall resulted and following the injection of this salt, a genital substance was released with force from all five pores. At the time of this observation, he devised a procedure for obtaining sea urchin gametes. He also found that a drop of isotonic calcium chloride on a gonad surface appeared to cause the wall to contract and relax in a rhythmical manner and the injection of this salt to cause characteristic rhythmical shedding followed by the complete release of genital substance from the gonads. In the preceding papers, γ-aminobutyric acid (GABA) was found to induce rhythmical as well as phasic contraction of the sea urchin gonad and gamete shedding [2, 3]. This prompted the authors to examine again gonad rhythmical contraction using calcium. In the present study, measurements were made of rhythmical contraction induced by calcium. Calcium, potassium, acetylcholine (ACh) and GABA were compared for their ability to bring about gonad response.

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MATERIALS AND METHODS

Animals

Sea urchins (*Strongylocentroturs intermedius*) were collected from the coast of Rishiri Island off northern Hokkaido during all seasons of the year. The animals were kept at our laboratory in an aquarium provided with running seawater until use.

Seawater

Modified van't Hoff seawater (ASW) (462 mM NaCl; 9 mM KCl; 9 mM $CaCl_2$; 36 mM $MgCl_2$; 17 mM $MgSO_4$; 20 mM Tris-HCl, pH 8.2) was used as the basal incubation medium. Ca^{2+}-rich ASW was prepared at various concentration of Ca^{2+}, using $CaCl_2$ in place of NaCl to maintain isotonicity. K^+-rich ASW was prepared in a similar manner. ACh (Sigma Chemical Co.) and GABA (Nakarai Chemicals Ltd.) seawater solutions were made by the addition of various concentrations of each chemicals to ASW.

Apparatus

The apparatus used in the present study is described in the preceding paper [2].

Recording

The recording was carried out at room temperature which varied from 18 to 25°C.

To measure gonad contraction, namely potential difference (PD) devised by Okada *et al.* [4], a hole 3 cm in diameter was made on the oral side of each sea urchin with solid scissors followed by the removal of Aristotle's lantern and the oesophagus by forceps. The animal was subsequently fixed by pinching the test with large forceps and immersed up to its equator in filtered natural seawater in a beaker and soon after, the body cavity was filled with ASW. The straw previously cut vertically in half and connected to a strain guage (SB–1TH, Nihon Koden) was placed on a gonad and the siphon in the central portion of body cavity. The gonad was allowed to remain in this state for 30 min; during which time the ASW was changed two or three times. The gonad was first treated with a stimulant at a concentration of x mM until immediately following a peak of induced contraction (Fig. 1). The gonad was washed, placed in ASW solution and 30 min later, the same treatment was repeated but at a stimulant concentration of y mM (Fig. 1). This was followed by washing the gonad and placing it in ASW for 30 min. It was finally treated with 500 mM K^+ ASW. Mechanical response by 500 mM K^+ was designated as $PD_{500K\ or\ K}$ The relative value of PD_{St} (stimulant) against PD_K was represented by relative response (RR). In this way, the two RRs of one preparation were measured.

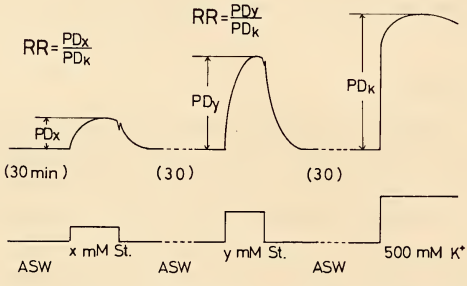

FIG. 1. Method for measuring relative response (RR). In a gonad, two RRs were measured at different concentrations of a stimulant. Immediately after PD peak was reached, the gonad was washed and immersed in ASW for 30 min. ASW, artificial sea water; PD, potential difference; St., stimulant.

RESULTS

Ca^{2+} effects

Dose-response experiment This experiment was conducted in March. The average weight of one lobule in a gonad was 0.28 g and from external observation, it appeared to be in the resting state. The first recording was generally performed at a low Ca^{2+} concentration and the second, at a high concentration. The filled circles in Figure 2 indicate the occurrence of rhythmical contraction superimposed on phasic one. The highest peak of rhythmical contraction was measured. The Ca^{2+} concentration inducing a threshold-contraction was about 20 mM. At about 100 mM, the dose-response curve indicated saturation of gonad response to Ca^{2+} with an RR of 0.74. The 50% effective dose ranged from 30 mM to 40 mM Ca^{2+}. For generation of rhythm, the threshold-contraction was induced at a concentration of 40 mM Ca^{2+}.

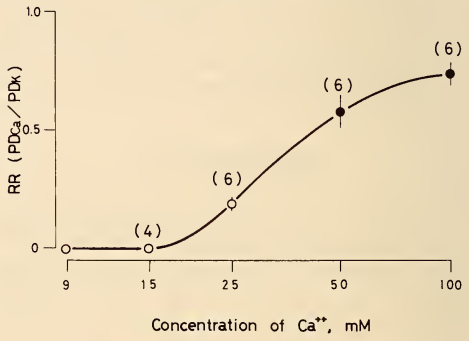

FIG. 2. Dose-response curve of potential difference (PD) by Ca^{2+}. Filled circles indicate rhythmical contraction. Points and vertical bars are means ± SEM. The number of preparations assayed appears in the parentheses. Abscissa, concentration of Ca^{2+} (mM); ordinate, relative response (RR).

Contraction mode Figure 3 a and b were recorded using the same ovary. The sea urchin

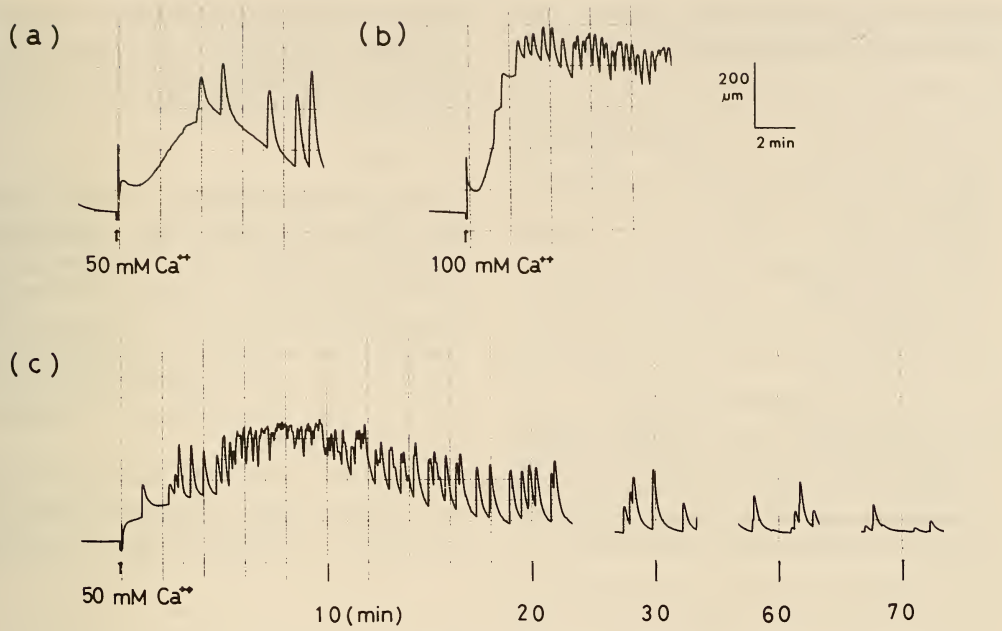

FIG. 3. Several features of PD by Ca^{2+}. Frequency of rhythm was noted to rise with increase in Ca^{2+} concentration. (a) 50 mM Ca^{2+}; (b) 100 mM Ca^{2+}. All time course of rhythmical contraction by 50 mM Ca^{2+} is shown in (c). The rhythm continued for 70 min.

from which it came spawned in response to over 50 mM Ca^{2+}. The frequency of the rhythm increased with Ca^{2+} concentration. The initiation of rhythm occurred more quickly at 100 mM than at 50 mM. Rhythm was greater at 50 mM than at 100 mM, since, owing to its high frequency at 100 mM Ca^{2+}, it became superimposed on itself. All the time courses of rhythmical contraction induced by 50 mM Ca^{2+} are shown in Figure 3c. The highest frequency was noted within 6–8 min. The contraction continued for 70 min following immersion of the gonad in the Ca^{2+}-rich ASW and then ceased. Generally, the rhythm in young gonad did not occur easily.

K^+ effects

Dose-response experiment The experiment was carried out in March. The average weight of one lobule was 0.34 g and the gonads appeared to be either in the resting or young stage. At 100 mM, gonad RR was maximium (Fig. 4). The threshold concentration ranged from 9 mM to 15 mM, with the 50% effective dose being 30 mM. Response difference according to sex could not be detected.

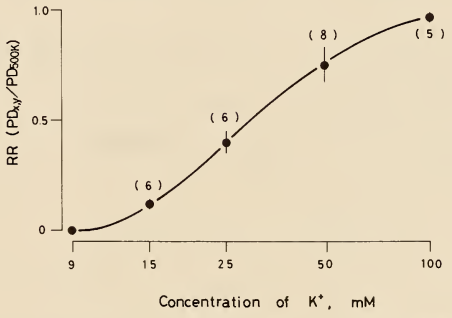

FIG. 4. Dose-response curve of potential difference (PD) by K^+. Points and vertical bars are means ± SEM. The number of preparations assayed appears in parentheses. Abscissa, concentration of K^+ (mM); ordinate, relative response (RR).

Contraction mode Although, when a few drops of 0.5 KCl were added to coelomic fluid, the sea urchin gonad induced rhythmical contraction [4], K^+-rich ASW prepared at various concentrations of K^+ generally failed to produce any rhythm. Contraction due to K^+ at concentration exceeding 100 mM was noted to be quite active but

to be followed by a state of rigor. That is, gonad relaxation was rendered impossible by K^+ treatment. The contraction produced by K^+ at 50 mM is shown in Figure 6a. To bring about the relaxation of the gonad, one hour was required. No rhythm could be observed.

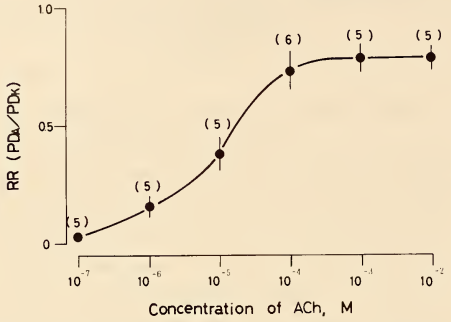

FIG. 5. Dose-response curve of potential difference (PD) by ACh. Points and vertical bars are means ±SEM. The number in parentheses is number of preparations assayed. Abscissa, concentration of ACh (M); ordinate, relative response (RR).

ACh effects

Dose-response experiment This experiment was performed in March. The average weight of one lobule was 0.27 g. The gonads appeared to be in the resting stage. The threshold contraction-concentration was 10^{-8} to 10^{-7} M (Fig. 5). RR at concentrations exceeding 10^{-4} M ACh was saturated at a value of 0.8. The 50% effective dose was about 10^{-5} M. Sex difference could not be detected.

Contraction mode No rhythmical contraction occurred at any ACh concentrations used. Relaxation of contraction by high ACh concentra-

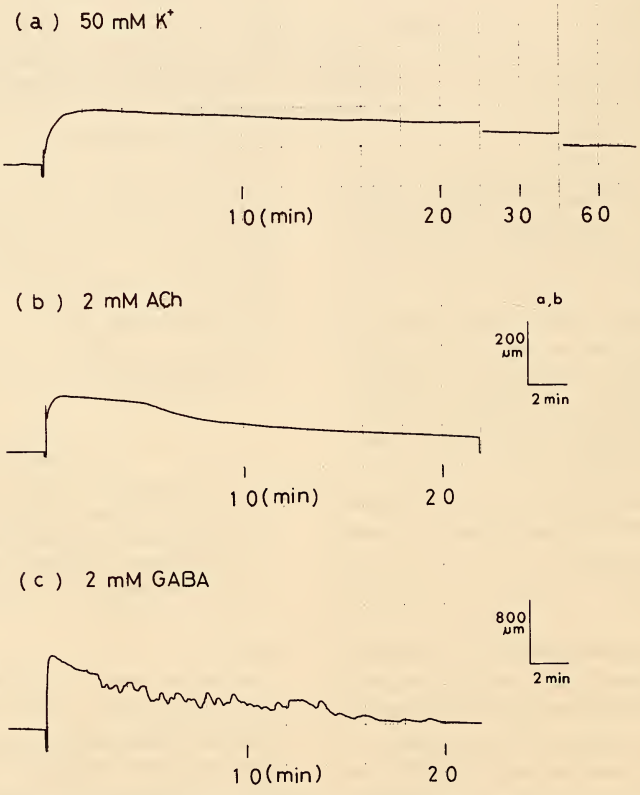

FIG. 6. Potential difference (PD) for each of three stimuli. (a) 50 mM K^+; (b) 2 mM ACh; (c) 2 mM GABA. Time course of PD in 50 mM K^+ was about one hour and that of ACh and GABA (2 mM) was about 20 min.

tion (2 mM) was possible, as evident from Figure 6b. The gonad was a testis and sperm continued to be released for 15 min. ACh contractions generally terminated within 20 min.

GABA effects

Dose-response experiment The data presented here have already been presented in the preceding paper [2]. Dose-response is shown in Figure 7. The threshold, 50% effective, and maximal doses were 0.01 mM, 0.1 mM and 10 mM GABA, respectively.

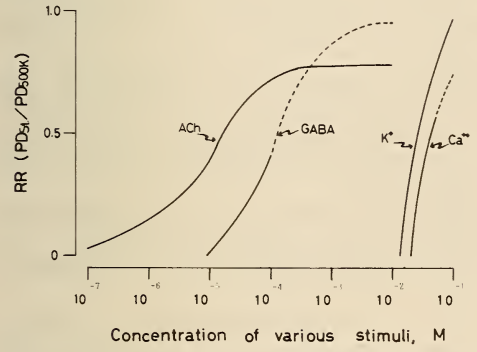

FIG. 7. Dose-response curves of potential difference (PD) by Ca^{2+}, K^+, ACh and GABA, on the same co-ordinate. Dotted lines indicated rhythmical contraction. Abscissa, concentration of stimuli; ordinate, relative response (RR).

Contraction mode The type of GABA contraction has already been presented in Figure 4 of the preceding paper, but in the present research as well, the contraction was found to be rhythmical (Fig. 6c). This was noted in July. Response to GABA ceased in 20 min.

DISCUSSION

Ca^{2+} response

Palmer [1] found that, on placing a drop of isotonic calcium chloride on the surface of a gonad of *Arbacia punctulata*, its wall appeared to contract and relax rhythmically. The data of the present research confirm this observation. The rhythmical contraction of a gonad of *Strongylocentrotus intermedius* was recorded with an oscillograph. Also, in his investigation, following an injection of $CaCl_2$, a definite relation between Ca^{2+} concentration and both the time prior to visible shedding and duration of the shedding reaction could be clearly discerned. In the present study, rhythm frequency was observed to increase with Ca^{2+} concentration. The rate of increase in PD was also found to do the same. In regard to gamete shedding in response to Ca^{2+}, Palmer found the average time interval between an injection of $CaCl_2$ and gamete shedding to depend on sex type. The time of sperm shedding for a 1 ml injection of 0.3 M $CaCl_2$ was 18 sec and that of egg shedding, 95 sec. In the present *in vitro* experiments, such variation according to sex could not be detected. Threshold concentration and rate of increase in calcium contraction were the same for both male and female. Rhythmical contraction also occurred at the same concentration (50 mM) in both sexes. The absence of any variation according to sex may possibly have been due to the conditions of the *in vitro* experiment and/or individual species differences.

Comparison of response to Ca^{2+}, K^+, ACh and GABA

Dose-response curves for Ca^{2+}, K^+, ACh and GABA appear on the same co-ordinate in Figure 7. The dotted line indicates rhythmical contraction. The threshold concentration of ACh is lower than that of the others. RR in the case of GABA and K^+ was as much as 1.0 but that in Ca^{2+} and ACh, 0.8. The duration of response to K^+ and Ca^{2+} was in proportion to their concentration and more than an hour. But for GABA and ACh, the duration was about 20 min. GABA and Ca^{2+} induced both rhythmical and phasic contraction but K^+ and ACh, only slow phasic or long lasting contraction.

The gonad displayed at least two kinds of contraction with or without rhythm. Consequently, it may be considered that contractions are produced by at least two different types of generators, one inducing smooth phasic and the other, rhythmical contraction. The present data show the first type of generator to be influenced by K^+, ACh and GABA and the second, by Ca^{2+} and GABA. Generation in one case appears to involve stable reactions with various stimuli regardless of

the season but the latter is apparently influenced by season since the number of such reactions was noted to increase with gonad maturation. This confirms the possibility of at least different types of generators.

Certain muscles of echinoderm are known to be responsive to ACh. In the lantern retractor muscle of the sea urchin, ACh at a concentration of 10^{-5}M has been found almost invariably to produce powerful contraction which persists with hardly any change for at least 80 min [5]. Moreover, the sea urchin oesophagus has been shown to contract through the action of ACh. Thus, although neuro-muscular transmission in the sea urchin is generally considered cholinergic, the gonad wall responds to GABA as well as ACh [2, 7]. The wall is composed of three layers, outer coelomic epithelium (visceral peritoneum), middle connective tissue with muscle and nerve cells, and an inner germinal layer [8]. The middle layer is further divided into five sub-layers, outer connective tissue, a muscle layer, central connective tissue, a nerve plexus, and inner connective tissue. Davis [9] showed nerve cell processes are present between the visceral peritoneum and the outer connective tissue. Thus, the muscles constitute one layer but the nerves, two layers in the gonad wall of the sea urchin [2]. The one muscle layer may possibly be controlled by various types of nerves whose determination should be made so as to gain an understanding of the spawning mechanism in the sea urchin.

ACKNOWLEDGMENTS

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